UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES
Antimicrobials Division

May 10, 2002

MEMORANDUM:

Subject: Efficacy Review EPA Reg. No. 63838-01 Bioside HS 5%
DP Barcode 280201
Case No. 064697

From: Nancy Whyte, Microbiologist NW
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510C)

To: Marshall Swindell/Tony Kish
Regulatory Management Branch I
Antimicrobials Division (7510C)

Thru: Emily Mitchell, M.S., Team Leader Emily Mitchell 5/14/02
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510C)

Thru: Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510C)

Applicant: Enviro Tech Chemical Services, Inc.
PO Box 577470
Modesto, CA 95357

Formulation Label:

Active Ingredient(s)	**% by wt.**
Hydrogen peroxide	26.5%
Peroxyacetic Acid	4.9%
Inert Ingredients	68.6%
Total	100.00%

I. Background:

The registrant has submitted two new efficacy studies to demonstrate the effectiveness of this disinfectant/sanitizer against *Listeria monocytogenes* and *Salmonella choleraesuis*

(MRID No. 455764-01 and 455764-02) to replace two studies submitted previously. The studies being replaced tested the same two organisms against a dilution of the product at 1.5 oz./5 gallons water. The new studies were conducted at a dilution of 1 oz./5 gal. water. .

II. Use Directions:

The draft label submitted in the current package lists usage of this product in many areas including precleaned surfaces in dairies, breweries, wineries, beverage and food processing plants, egg processing and packing equipment surfaces, and eating establishments. It is effective in hard water up to 400 ppm.

For use as a food-contact sanitizer, the product is diluted 1 ounce in 5 gallons water. Surfaces should be precleaned to remove gross food particles, washed with detergent, and rinsed with potable water prior to treatment.. The product may be applied by a coarse spray or circulation techniques as appropriate. The surfaces must be exposed to the product for at least 60 seconds or more if required by the governing code. and allowed to air dry without rinsing. At this dilution it is effective against *Escherichia coli, Listeria monocytogenes,* and *Salmonella choleraesuis.* Use dilutions up to 2.6 oz of the product per 5 gallons water may be used without a potable rinse, but dilutions above this amount must be rinsed with potable or sterile water, or by using a sanitizing rinse (1 oz./5 gallons). These same directions are used to sanitize eating, drinking, and food preparation utensils. All utensils should be immersed in the solution for 60 seconds, drained, and allowed to air dry. A solution of 1-1.5 oz in 5 gallons of water is also used as a final sanitizing rinse for returnable and non-returnable bottles.

To sanitize conveyors and equipment for meat, poultry, seafood, fruit and vegetables a solution of the product at a dilution of 1-1.5 oz./5 gallons of water is used. All surfaces should be exposed to the solution for at least 60 seconds and allowed to drain dry.

A solution of 2-20 oz. of product in 5 gallons (0.3-3.0%) may be used for fogging rooms and packaging areas. One quart of this solution is adequate for 1000 cu ft. of room area. All personnel must leave the area prior to, during, and after fogging until the hydrogen peroxide concentration is below 0.5 ppm, or there is no strong odor of acetic acid present. Surfaces must be allowed to drain thoroughly before operations in the area are resumed. For use in fogging potato storage sheds where harvested potatoes are kept, a solution of 1 gal. of product per 4 gallons water is used. This method is for use on potatoes that will be peeled or further water-processed.

When used in low-temperature machines for sanitizing tableware, a solution of 1 oz/5 gallons is injected into the final rinse water, followed by air drying. The rinse solution should be tested with a test kit periodically to insure that the concentration does not fall below 0.1%.

Prepared use solutions for mechanical operations may not be reused for sanitizing, but may be reused for cleaning. For manual operations, fresh sanitizing solutions must be prepared daily or more often if they become diluted or soiled.

When used as a combination disinfectant/cleaner on hard non-porous surfaces the product is effective in hard water (400 ppm) and in the presence of 5% organic soil load in healthcare institutions, industrial establishments, schools and other educational facilities, office buildings, recreational areas, and poultry premises and equipment. Heavily soiled areas must be pre-cleaned and followed by a potable water rinse. The solution may be applied with a mop, sponge, cloth, brush or similar method, or by soaking, spraying, or immersion. The areas treated must remain wet during the 10 minute exposure time. Solution and entrapped soil may be removed with a clean wet mop, cloth, wet vacuum pickup, or by draining. No rinsing should be done.

For fruit and vegetable washes a solution of 1 oz. in 5 gallons of water is used by

spraying or immersion. Exposure time for this use is one minute, and draining is needed. There is no need for a rinse following treatment.

When used to sanitize ultra filtration and reverse osmosis membranes and associated piping systems this product can be added continuously to the feed water stream at a rate of 1% by volume. Incompatible equipment such as carbon filters and ion exchange equipment must be removed before treatment. Cleaning and rinsing with potable water must be done prior to treatment at 20° C for at least 10 minutes or up to 4 hours. After recirculation is complete, the system must be rinsed with potable or RO water until residue hydrogen. peroxygen concentration is below 3 ppm.

To control slime in cooling water systems and bacteria and fungi in dispersed pigments, an amount of 4-20 oz. of product/1000 gallons of solution is used as a continuous or intermittent slug treatment or in the manufacture and storage of pigments such as clay and other natural materials used in paint and paper products.

III. Agency Standards for Proposed Change:

The Agency standards for food-contact sanitizers are found in DIS-TSS-4 which specifies that data must be generated by use of the *Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC),* Official Method 966.09 Germicidal and Detergent Sanitizing Action of Disinfectants, current edition. AOAC, Arlington, VA. Initial testing of the product is required against *Escherichia coli* and *Staphylococcus aureus.* One test with one sample from 2 different batches must be conducted for additional organisms for which effectiveness claims are made. If claims are made for effectiveness in hard water, all required data must be collected using hard water. Acceptable results must show a 99.999% reduction in number of microorganisms within 30 seconds. Results must be reported as the actual count and the percentage reduction over the control.

IV. Summary of Submitted Study:

The efficacy studies submitted with this application to support additional label claims were conducted by Gibralter Laboratories, Fairfield, NJ in July 2001, using Good Laboratory Practices and following the AOAC suspension test standards required by Agency. The organisms tested were *Listeria monocytogenes* ATCC 984 and *Salmonella choleraesuis ATCC 10708.* The same two lots of the product, 28545/1 Lot #10206 manufactured Dec.6, 2000 and 24545/2 Lot #10102 diluted 1 ounce in 5 gallons (1: 640, 78 ppm) AOAC hard water, were used in testing against both organisms. The expiration date of the product was not known. Other than the statement that test organisms were prepared using Brain-Heart Infusion agar (BHIA), no other information about numbers of subcultures done prior to testing and confirmation of identity of organisms was provided in the study description. Tests were not conducted in the presence of an organic soil load.

The diluted test material was aliquoted into two wide-mouth erlenmeyer flasks, using 99 ml in each flask. The flasks were placed in a 20 +/- 0.2° C water bath and allowed to equilibrate for ≤20 minutes. Two additional flasks of sterile phosphate buffer dilution water were prepared in a similar manner to serve as numbers control. The efficacy assay was performed by adding 1 ml of the test organisms to the product flasks as per AOAC. The number of organisms present in the flasks were determined at both 30 and 60 seconds. Ten-fold serial dilutions were made in trypticase soy broth with 4.0% Tween 20 and 0.5% azolectin (GBL STAT broth Lot # F-196). Pour plates were performed in quadruplicate using BHIA containing I ml of AOAC hard water and phosphate buffer dilution water as a neutralizer.

Plates were incubated for 48 hours at 37° +/-1C. Colony-forming units were counted using a Quebec colony counter.

The number controls were performed by adding 1ml of the test organisms to the two wide-mouthed flasks containing sterile phosphate buffer dilution water. The number of bacteria present were determined at ≤ 30 seconds. Ten-fold serial dilutions were made in 9 ml. GBL Stat broth. Pour plates were made in quadruplicate and incubated and counted as with the test plates. Sterility controls of the neutralizer broth were done as with the number controls, but the flasks were not inoculated. After colonies were counted, surviving colonies, if any, from pour plates were subcultured and incubated 24-48 hours at 37° +-1C to confirm typical colonial morphology. Both lots of product from both flasks showed no growth of either organism following exposure to the product for 30 seconds or 60 seconds after 48 hours incubation. The number of organisms initially present before treatment (control) are as follows:

Listeria monocytogenes ATCC 984		*Salmonella choleraesuis* ATCC 10708	
Lot #10102 and Lot #10206			
Flask A	72, 90, 73, 72	Flask A	88, 91, 65, 74
Flask B	83, 67, 65, 77	Flask B	98, 89, 81, 84

Calculated results are as follows:

Listeria monocytogenes	*Salmonella choleraesuis*
Aver. no surviving (cfu/ml) <10	Aver. no. surviving (cfu/ml) <10
No. initially present (cfu/ml) 7.5×10^7	No. initially present (cfu/ml) 8.4×10^7
No. initially present (Log_{10}) 7.87	No. initially present (Log_{10}) 7.92
Log reduction ≥ 6.87	Log reduction ≥6.92
Percent reduction ≥ 99.999%	Percent reduction ≥99.999%

V. Labeling:

1. The directions on page 3 of the draft label in the first complete section of that page "Sanitization....." state a dilution of 1.5 ounces/gal. for control of *Listeria monocytogenes* and *Salmonella choleraesuis*. These organisms were proved effective at 1 ounce/5 gallon, so the concentration may be decreased for this use.

VI. Comments and Recommendations:

1. The product, *Bioside 5%*, diluted to a concentration of 78 ppm in AOAC hard water, demonstrated effectiveness as a food-contact sanitizer against *Listeria monocytogenes* ATCC 984 and *Salmonella choleraesuis* ATCC 10708 when exposed for 30 seconds at 20° +/- 1C using the AOAC suspension test, reducing the number of organisms by ≥99.999%

2. No organic soil load was used. Surfaces to be treated must be pre-cleaned prior to treatment.

3. Laboratory procedure should more fully describe preparation of organisms for testing, e.g., method used to store organisms, number of subcultures before day of testing, size of inoculum, method of verifying pure cultures.